Formation of Perforation Plates and Bordered Pits in Differentiating Vessel Elements

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Shigeki YATA*, Takao ITOH** and Tsuneo KISHIMA**

Abstract——Disintegration processes of end walls and pit membranes in later stage of maturation of the vessel elements were observated with electron and optical microscopes using a poplar tree (*Populus nigra* L. var *italica* KOEHNE).

The major components of the end walls may be disintegrated enzymatically from their surfaces, but disintegration of the minor components (probably a part of cellulose microfibrils) may be achieved by the transpiration streams.

In the case of intervessel pit membranes, it seems that only the non-cellulosic substances may be removed enzymatically. Parenchyma cells adjacent to vessels, having protective layers, seem to be got rid of the enzymatical attack through the vessel-parenchyma pit membranes.

Considering the observation results by O'BRIEN (1970)[6], it seems likely that the disintegration of unlignified cell walls is a general phenomenon that occurs later in the autolysis of the vessel elements.

Introduction

Vessels are the main passage of sap in hardwood trees and also play an important role in penetration of liquid into wood for various treatment processes. Anatomical features of the vessel elements concerning penetration are their larger diameters and lack of their end walls.

Among the previous informations on the formation of vessels, the structure of end walls in differentiating vessels was precisely studied by K. ESAU (1936, 1940)[1,2] in optical microscope level. She made clear the following facts, that is, (1) during rapid expansion of vessel elements, their end walls became thicker and more conspicuous than their longitudinal walls, (2) during secondary wall thickenings of longitudinal walls, the end walls did not accumulate secondary walls, and they were composed of two primary walls and an intercellular layer, and (3) after the secondary wall thickening completed, the end walls began to break down.

But the exact feature of the process of the disappearance of end walls during the development of their perforations has not been known yet. According to the assumption of P. A. ROELOFSEN (1959)[3], both cellulosic and non-cellulosic components in the end walls are removed by the activity of the protoplast of the

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vessel elements, but, A. FREY-WYSSLING (1959)[4] has suggested that only non-cellulosic component is removed and cellulosic microfibrillar networks are pushed from the original position to the margin of perforations. R. BUVAT (1964)[5] has found that thicker end walls bear fibrous layers on both sides, which are continuous with primary layers of the side walls. He interpreted these fibrous layers as the cellulosic component and the enclosed clear layers as the pectic one. And, T. P. O'BRIEN (1970)[6] has supposed that many components of the end walls would be hydrolyzed by hydrolases which is released during autolysis of protoplasts.

In this paper, the decomposition processes of the end walls accompanying to the maturity of vessel elements were examined, and besides, some structural changes of intervessel pit membranes and vessel-parenchyma pit membranes were also discussed.

Materials and Methods

Materials of new shoots including cambium and differentiating xylem were obtained from rapidly growing poplar (*Populus nigra* L. var. *italica* KOEHNE). For electron microscopy, the materials were fixed, immediately after cutting, with 3 % glutalaldehyde in 0.2M phosphate buffer at pH 7.2, washed with the same buffer solution, and postfixed with 1 % osmium tetraoxide in the same solution. A part of the materials was fixed with 1 % potassium permanganate. After dehydration in an ethanol-propylen oxide series, the materials were embedded in epoxy resin and sectioned on a Porter Blum MT-1 ultramicrotome using glass knives. The sections were stained with uranyl acetate and lead citrate. For optical microscopy, the materials were fixed in FAA solution, embedded in epoxy resin, and sectioned at 1~5 μ thickness. Then the sections for electron microscopy were observed in a JEM-T6S electron microscope.

Results

1. *Formation of Simple Perforation Plates in Secondary Xylem*

Perforation plates of vessel elements in secondary xylem of poplar wood were the simple one. During lateral growth, the end walls became conspicuously thicker than the other parts of the walls showing the typical three layered structure, i.e., primary wall—intercellular layer—primary wall (Fig. 1). In the end walls, both primary wall and intercellular layer seemed to be thickened. Rarely plasmodesmata were observed in the end walls. After the rapid surface growth has been completed, secondary wall layers deposited but the portions of end walls to be perforated were not covered with the wall substances.

After the secondary walls has been fully formed and lignified, protoplast dis-

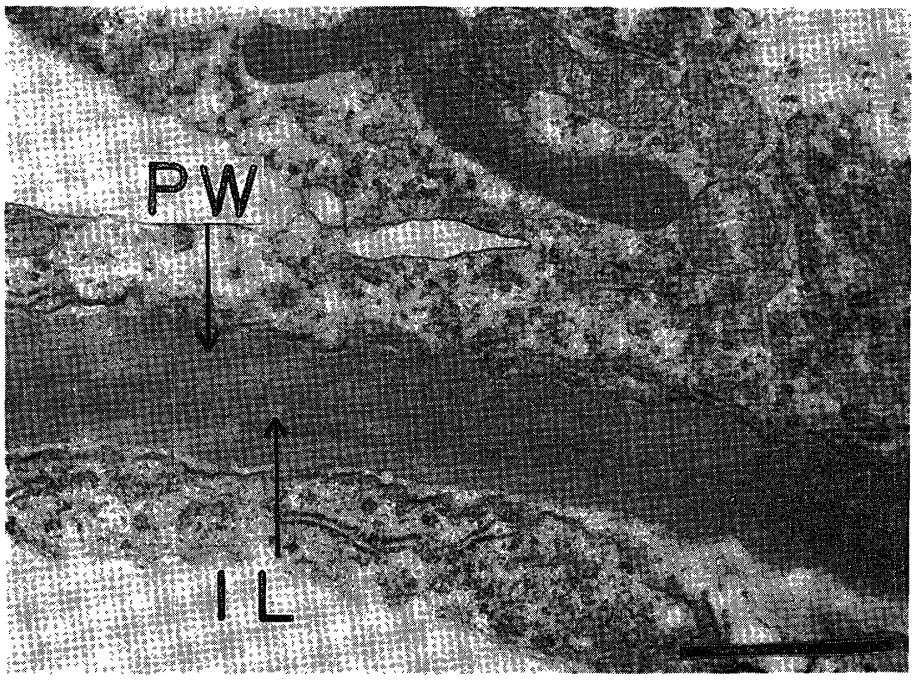

Fig. 1. A part of the thickened end wall in a differentiating vessel element showing an intercellular layer (IL) and two primary walls (PW) on both sides. Scale : 2 μ.

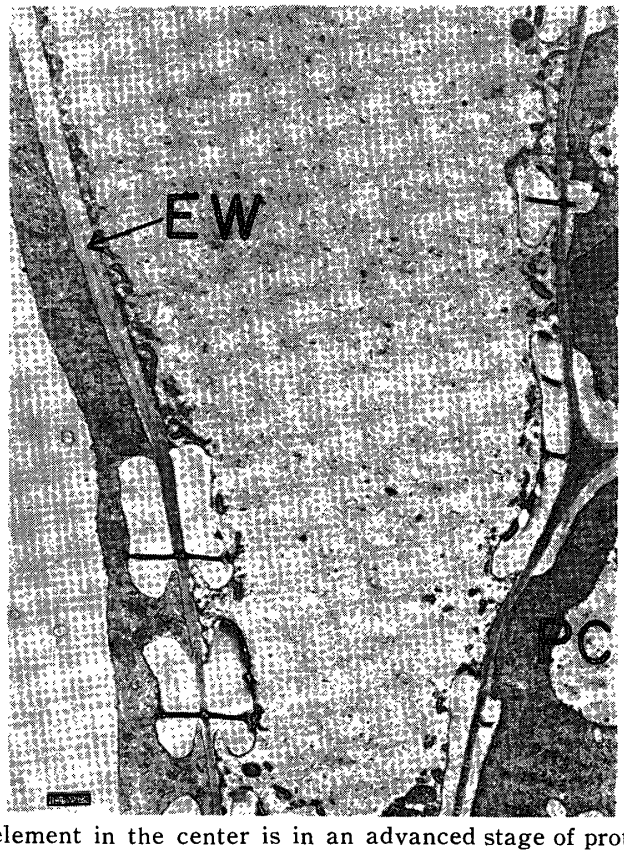

Fig. 2. The vessel element in the center is in an advanced stage of protoplast degradation than an adjacent vessel element to the left. End wall (EW) is not disintegrated and parenchyma cell (PC) adjacent to the autolyzing vessel element is forming no protective layer. Scale : 2 μ.

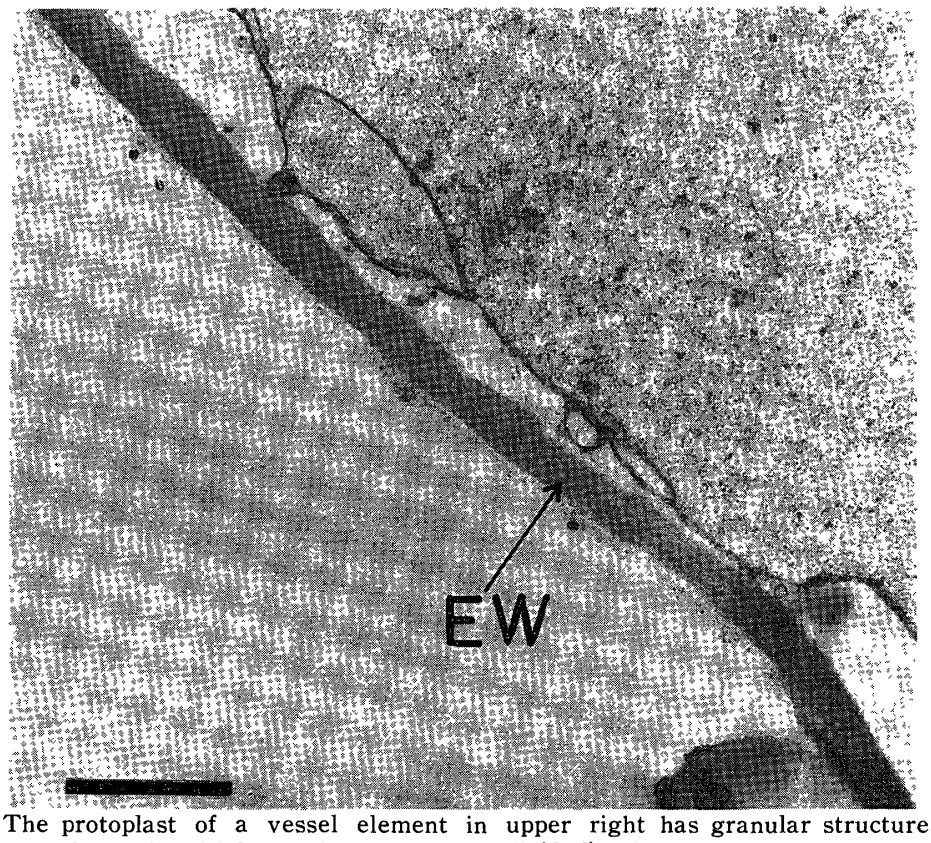

Fig. 3. The protoplast of a vessel element in upper right has granular structure and the plasma membrane is withdrawn from the end wall (EW). In the adjacent vessel element in lower left, the protoplast is lost and the end wall is disintegrating on this side. Scale : 2 μ.

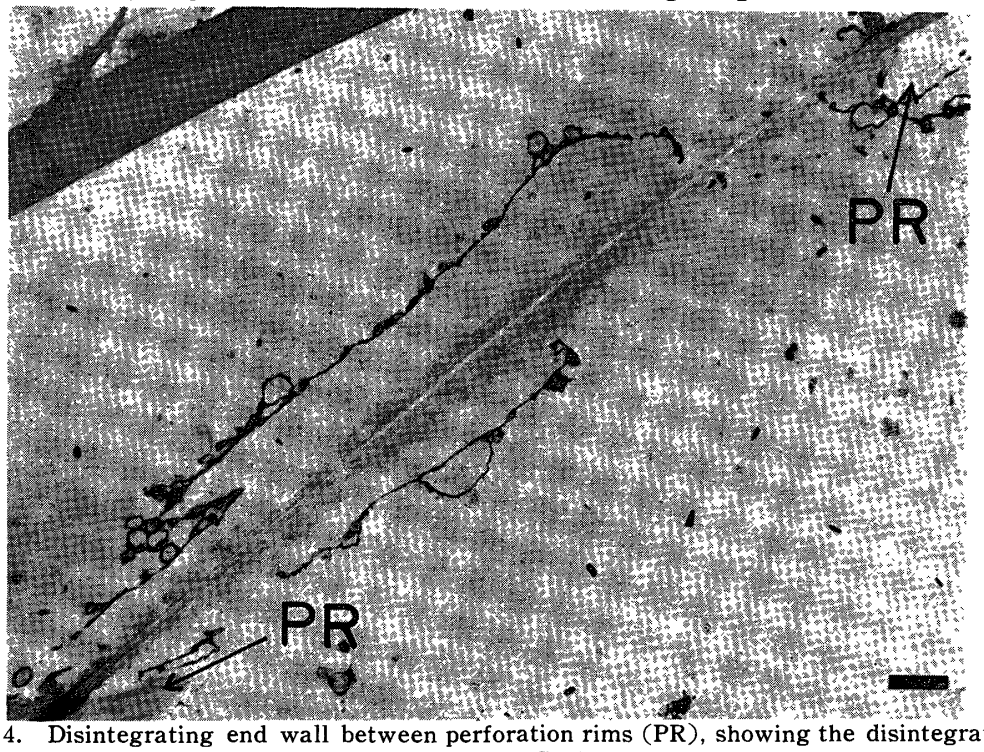

Fig. 4. Disintegrating end wall between perforation rims (PR), showing the disintegration proceedings on both surface of the end wall. Scale : 2 μ.

ruption was started by autolysis of cells, that is, the organelles were deformed and decreased in number and protoplasts began to show granular structure (Fig. 2). In advanced stage, protoplasts were scarcely observed and plasma membranes

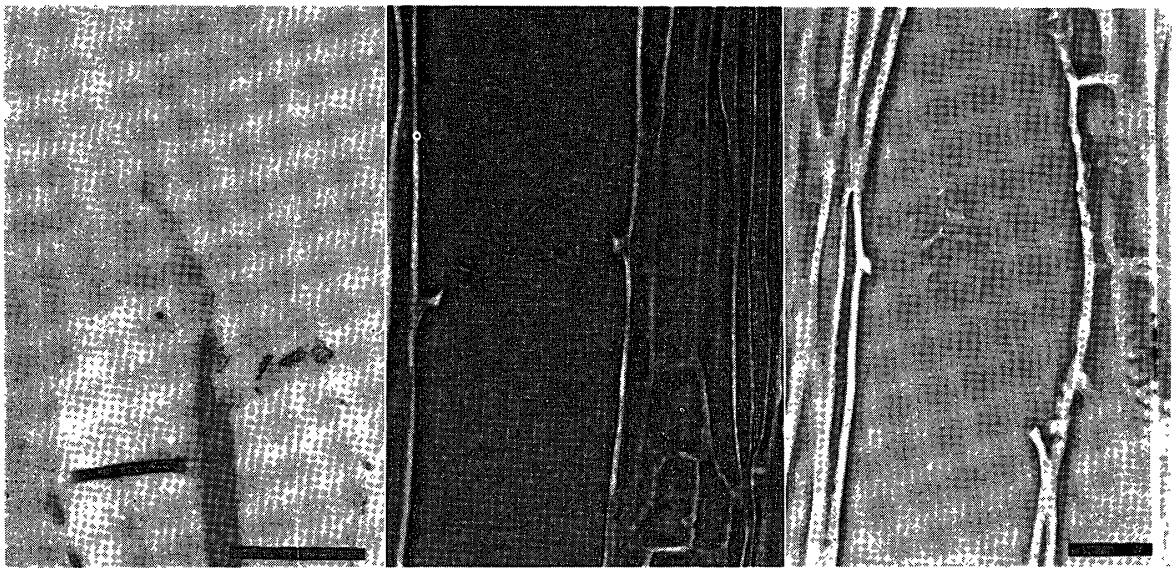

Fig. 5~7. Traces of end walls between vessel elements immediately after the completion of perforations. Scale : 2 μ···Fig. 5, 20 μ···Fig. 6 and 7.

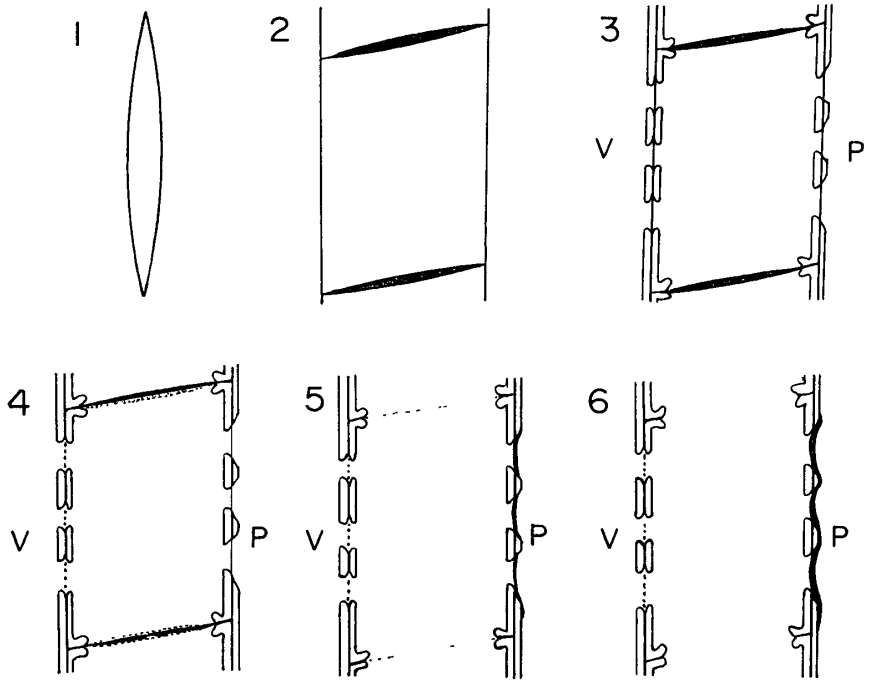

Fig. 8. A diagramatic representation of the development of perforation plates in vessel elements of poplar. P : adjoining parenchyma cell. V : adjoining vessel element.
1. Mother cell. 2. End walls thickened in surface growth stage. 3. No secondary wall formed on the end wall in secondary wall thickening stage. 4, 5. Decomposition stage of unlignified walls. Protective layers formed on the vessel-parenchyma pits. 6. Mature vessel element.

began to break down. And in this stage, decomposition of the end walls began also (Fig. 3). In this Figure, the cell at the upper right seems to be markedly decomposed. The plasma membrane presents but is separated from the original portion on the end wall. The end wall itself is more or less intact. On the other hand, in the cell at the lower left protoplast and plasma membrane are already lost and the end wall begins to decompose. Figure 4 shows the more advanced stage of decomposition in the end wall, and in this case, it is proceeding in both sides of the wall. Though protoplasts are not seen in the cells, traces of plasma membranes are observed. Decomposition is proceeding on the whole surfaces of the end wall.

Figs. 5~7 show perforation plates immediately after completion. A part of end wall components (perhaps cellulose microfibrils) is observed, but this is completely lost in mature vessel elements.

To make sure of the above described observation, a diagramatic representation

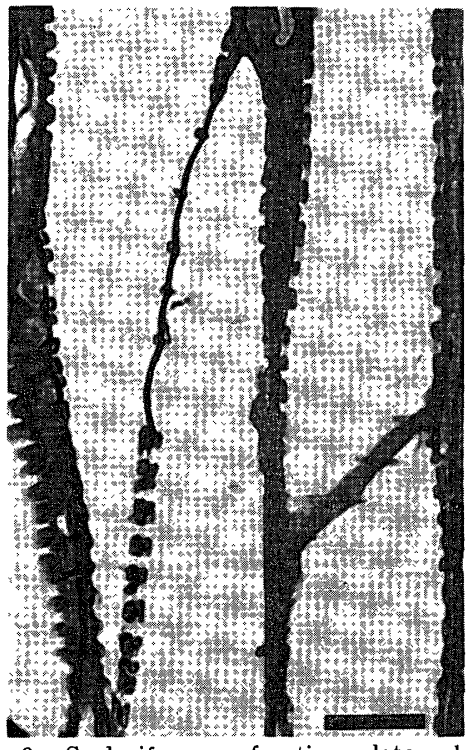

Fig. 9. Scalariform perforation plate, showing the intact end wall. Each bar of the perforation plates is formed on the end wall thickened. Scale : 20 μ. Optical micrograph.

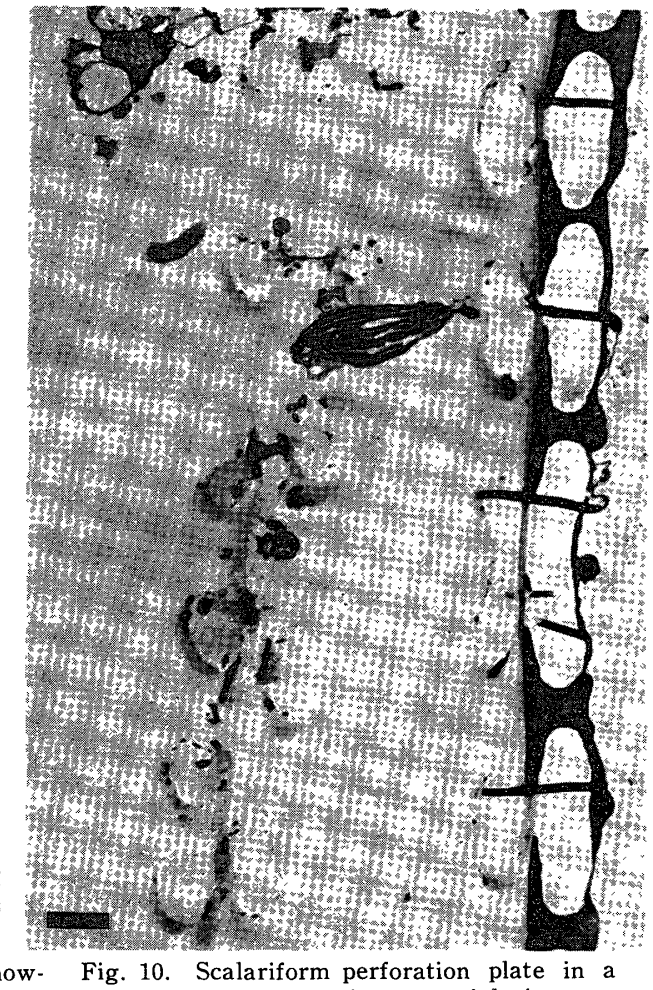

Fig. 10. Scalariform perforation plate in a mature vessel, showing a partial pit membrane remaining and some debris of protoplast on the plate. Scale : 2 μ.

of the development of a simple perforation is given in Figure 8.

2. Formation of Scalariform Perforation Plates in Primary Xylem

Perforation plates of vessel elements in primary xylem of poplar were originally the scalariform one in general, and the plates were inclined at small angles to the cell axes. The structure of end walls in primary vessel elements was the same as in secondary xylem, so each bar in the scalariform perforation plates was formed upon the thickened end walls (Fig. 9). Figure 10 shows the scalariform perforation plate which was formed immediately after the completion of perforations. Residues of protoplasts were accumulated in the vicinity of the perforation plates. A part of the end wall, presumably cellulose microfibrils, presented as a partial pit membrane between lignified thickenings, i.e., the perforation bars, which were lain close together in the plate.

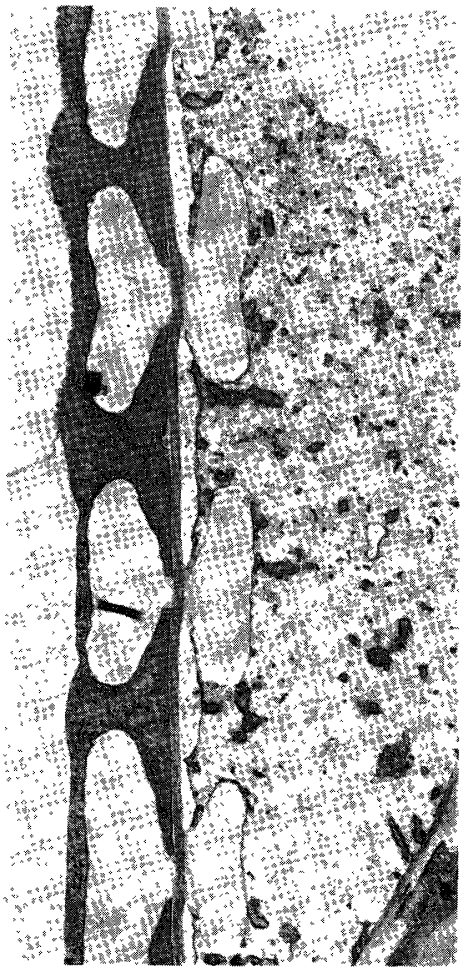

Fig. 11. Intervessel pits between autolyzing vessel element (right) and adjacent one (left). The pit membranes are not yet disintegrated.

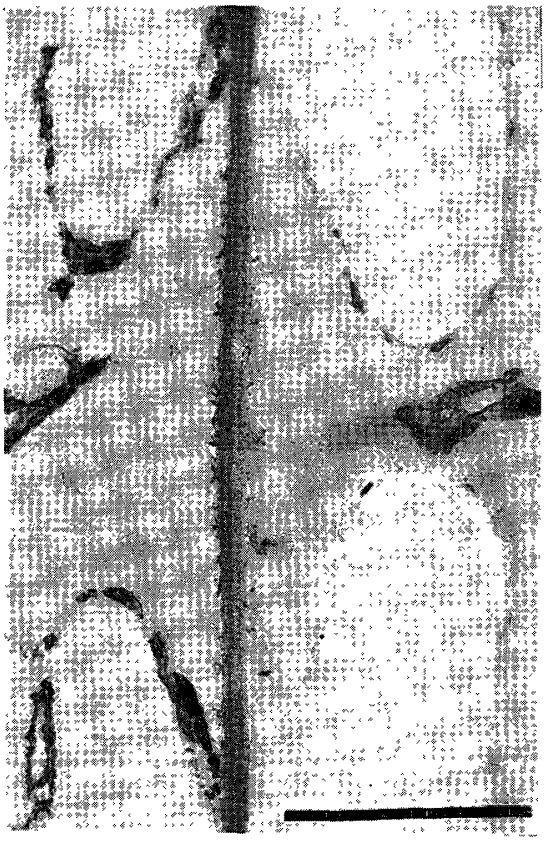

Fig. 12. Intervessel pits in a later stage of protoplast degradation. Pit membranes are disintegrating on the both sides of matured vessels. Scale : 2 μ.

Fig. 13. Pit membranes between matured vessel elements, showing disintegrated and mat-like structures of fine microfibrils. Scale : 2 μ.

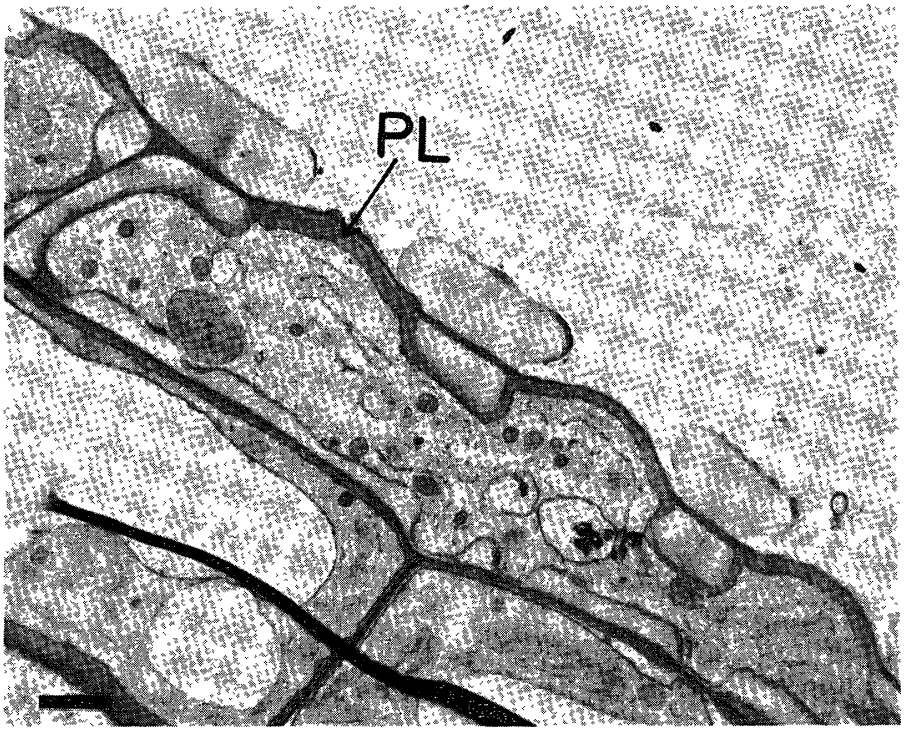

Fig. 14. Protective layers (PL) developed in parenchyma cells adjacent to a matured vessel element in primary xylem. Scale : 2 μ.

3. Decomposition of Pit Membranes in Bordered Pits

In the beginning of protoplast decomposition, intervessel pit membranes were left intact (Fig. 11) but they began to be decomposed in the later stage (Fig. 12). Disintegrated pit membranes seemed to consist of a mat of fine fibrils (Fig. 13). Consequently the materials which were removed from the pit membranes might mainly be matrix substances.

When vessel elements matured and protoplasts were degenerated, parenchyma cells adjacent to the vessel elements formed walls of a peculiar texture (Fig. 14). This additional wall was firstly described by R. SCHMID (1965)[7] as 'protective layer', meaning that the layer is to protect own parenchyma cell but not to protect pit membrane of adjacent vessel element.

4. Histochemical and Optical Tests of End Walls of Vessel Elements

In order to know chemical components of end walls of the vessel elements, the following histochemical and optical tests were carried out.

The end walls evinced only a very week double refraction between crossed Nichol's prisms which indicates the structure similar to cellulose. The walls were stained red with ruthenium red. After treatment with alkaline hydroxylamin/ferric chloride (ALBERSHEIM & KILLIAS, 1963), the end walls showed to be electron dense for pectic substances.

Tests for lignin with phloroglucinol-hydrochloric acid and potassium permanganate gave negative results. Toruidine blue-O which stains lignin blue did not show the end walls blue.

Summarizing these results, it is suggested that the end walls might be consist of mainly pectic substances and not be lignified, and that cellulose microfibrils were scarcely observed in spite of the thickened end wall.

Discussion

It became clear that the unlignified primary walls, that is, end walls and pit membranes of intervessel pits, are degenerated in the later stages of maturation of vessel elements, and the parenchyma cells adjacent to disintegrating vessel elements form protective layer in poplar.

It is reasonable to consider that these phenomena were brought about by polysaccharidases which were released in the autolyzing vessel elements. Observing the hydrolized walls in bean and wheat leaves and others, O'BRIEN (1970)[6] concluded that they were general phenomena that occured later in autolysis of all tracheary elements.

From this standpoint, the mechanism of autolysis is to be clarified. Cytological

and biochemical studies have established that lysosome might be concerned with the autolysis. In animal cells, it has been known that the breakdown of lysosome bring about release of hydrolase into protoplasm and results in autolysis of the cell (DIXON & WEBB, 1964)[9]. In plant cells, the information on the lysosome is very meager and its occurence could not be confirmed in this study. But it is probable that a certain organelle corresponding to the lysosome may be present in plant cells because of the actual occurence of protoplast disruption and the presence of decomposing membranes which is called hydrolyzed walls by O'BRIEN (1970)[6].

Next It comes into question why microfibrils in the end walls were removed and not so in the intervessel pit membranes.

As described previously, enzymatic disintegration of the end walls does not occur uniformly in all components but a part of the microfibrils still remains immediately after complete formation of the perforations (Figs. 6 & 7). But they are lost completely in the matured vessel elements, so it may be preferable to consider that the remunants are dispersed by the transpiration stream.

In the matured scalariform perforation plates, partial pit membranes are often present between bars which lie closely together in the plates. These partial pit membranes have been recognized by S. ISHIDA (1969)[10] and J. OHTANI (1970)[11] in KATSURA wood (*Cercidiphyllum japonicum* SIEB. at ZUCC.). Probably they could not be removed by the transpiration stream.

It seems that microfibrils in the end walls are ready to disperse in different from those in the intervessel pits, because of large diameters of perforation plates and large angles between end walls and the direction of tranpiration stream, i.e., cell axes. However, the above discussions are never compatible with the release of cellulase in autolyzing processes. It seems to be the most probable that, in the end walls, a part of cellulose microfibrils is degenerated by cellulase and the others are dispersed by the transpiration stream.

MEYER and CÔTÉ (1968)[12] recently found that protective layers were formed apparently by the process of typical cell wall growth in higher plants and resembled the secondary walls in appearance when matured. R. C. FOSTER (1967)[13] and MEYER and CÔTÉ[12] discussed the role of the protective layers with reference to the formation of tyloses.

In this observations using poplar, the protective layers were observed in the parenchyma cells adjacent to the matured vessel elements. If these additional layers appeared as a reaction of the parenchyma cells to the autolysis of the vessel elements, they should be observed in other hardwood species. Therefore, further studies should be conducted to whether the protective layers exist or not in many other species.

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